

## AMENDMENTS TO THE DRAWINGS

The attached five sheets of drawings include changes to Figs. 4G-4L, 5M-5T, 6A-6B, 7C-7N and 80-8T to change the Fig. numbers to Figs. 3G-3L, 3M-3T, 4A-4B, 4C-4N and 40-4T, respectively. These sheets 4/8 to 5/8 replace the previously filed sheets of drawings including sheets 4/8 to 5/8.

Attachment: Five Replacement Sheets

**REMARKS/ARGUMENTS**

Upon entry of this amendment, claims 1, 5-8, 12, 13, 16 and 17 will be canceled without prejudice or disclaimer of the subject matter recited therein so that claims 1, 2, 5-8, 12, 13, 16 and 17 will be canceled claims; claims 3, 10, 11, 14, 15, 18 and 19 will be amended, whereby claims 3, 4 and 9-11, 14, 15, and 18-21 will be pending. Claim 3 is an independent claim.

The claims have been amended to even more clearly denote the method of detecting cholesterol, as will be discussed in the response to the 35 U.S.C. 112 rejections below.

Reconsideration and allowance of the application are respectfully requested.

**Discussion Of Telephone Interview**

Applicants express appreciation for the courtesies extended by Examiner Jon Weber and Examiner Paul C. Martin during a September 6, 2007 telephone interview with Applicants' representative Arnold Turk.

During the interview, the various objections and rejections set forth in the Office Action were discussed. The Examiners agreed that the drawings and specification can be amended to renumber the Figures of drawings with respect to the indicated letters so that, for example, Fig. 4 will be labeled as Fig. 3.

Applicants' representative indicated that the specification would be amended to include reference to the PCT application, even though not required.

Amendment of claim 3 was discussed with respect to the 35 U.S.C. 112 rejections, and the examiners indicated that the claims should even more clearly recite the

material being detected, such as that labeled bound material is being detected.

Applicants' representative indicated that amendment of the claims would be considered.

Regarding the term "affinity", reference was made to Applicants' specification, and Applicants' representative indicated that further arguments would be presented in the response.

Regarding the prior art rejections, Applicants' representative briefly argued that the claimed subject matter was not inherently performed in Baba, and indicated that more detailed arguments would be presented in Applicants' response.

Arguments as presented during the interview are included in the remarks herein.

#### **Consideration Of Information Disclosure Statement**

Applicants express appreciation for the inclusion with the Office Action of an initialed copy of the Form PTO-1449 submitted with the Information Disclosure Statement filed December 18, 2006.

Applicants note that the Examiner has crossed through items 1, 2 and 16 asserting that copies have not been provided. In response, Applicants note that documents 1 and 2 are cited in the International Search Report, and this citation of the documents is noted in Applicants' Information Disclosure Statement. The Patent and Trademark Office should therefore have copies of these documents. However, to ensure that the record is complete, Applicants are submitting copies of these documents, i.e., L.F. AMOROSA et al., "The Effects of Polyoxyethylated Cholesterol Feeding on Hepatic Cholesterol Synthesis and Intestinal Cholesterol Absorption in Rats", *Atherosclerosis*, Vol. 64, pp. 117-123 (1987); and Hideki ISHIWATA et al., "Physical-Chemistry Characteristics and Biodistribution of

Poly(ethylene glycol)-Coated Liposomes Using Poly(oxyethylene) Cholesteryl Ether”, Chem. Pharm. Bull., Vol.43, No. 6, pp1005-1011 (1995). However, a copy of L.F. AMOROSA et al. is not presently available to the undersigned, and a copy is being obtained for submission.

Regarding item 16, i.e., Yiannis A. IOANINOU, “Multidrug Permeases and Subcellular Cholesterol Transport”, Nature Reviews: Molecular Cell Biology, Vol. 2, pp. 657-668 (2001), Applicants note that a copy of this document was submitted with the Information Disclosure Statement. Applicants are submitting another copy accompanied by a date-stamped mailroom receipt evidencing the filing of this document on December 18, 2006.

Moreover, upon review of the Form PTO-1449, it is noted that U.S. Patent No. 5,691,159 (which is listed as a family member of JP 8-131197 in the International Search Report) and Hideki ISHIWATA et al., Biochimica et Biophysica Acta, Vol. 1359, pp. 123-135 (1997) were inadvertently not listed on the form even though referenced in the Information Disclosure Statement.

In view of the above, Applicants are submitting herewith a Form PTO-1449 listing items 1, 2 and 16, U.S. Patent No. 5,691,159 and Hideki ISHIWATA et al., Biochimica et Biophysica Acta, Vol. 1359, pp. 123-135 (1997). The Examiner is requested to initial the form in the appropriate places, and to include an initialed copy of the form with the next communication from the Patent and Trademark Office.

Moreover, Applicants are submitting on even date herewith a Supplemental Information Disclosure Statement. The Examiner is requested to consider the

information cited in this Supplemental Information Statement along with the information noted above, and to make the information of record in this application.

Applicants are submitting herewith the fee of \$180.00 for consideration of this information. Authorization is also hereby provided to charge any necessary fee, including any fee under 37 C.F.R. 1.17(p), to Deposit Account No. 19-0089.

### **Response To Objection To Drawings**

The drawings are objected to in that the Office Action contends that labeled figures for each set of figures should be labeled beginning with the letter "A".

In response to this ground of objection and as discussed with the examiners during the above-noted telephone interview, replacement drawings are submitted herewith which have labeled the Figures of drawings on Sheets 4/4 to 8/8 from 4G-4L, 5M-5T, 6A-6B, 7C-7N and 8O-8T to Figs. 3G-3L, 3M-3T, 4A-4B, 4C-4N and 4O-4T, respectively.

In view of the submission of the Replacement Sheets of drawings, Applicants respectfully request approval of the drawings, and withdrawal of this ground of objection.

### **Response To Objection to Specification**

The specification is objected to for apparently not cross-referencing the PCT application.

In response, Applicants noted during the above-discussed telephone interview that amendment of the specification to include reference to the International Application is not necessary in a national stage application. In particular, it is not necessary for the

applicant to amend the first sentence of the specification to reference the international application number that was used to identify the application during international processing of the application prior to commencement of the national stage. See MPEP 1893.03(c)III., Rev. 5, August 2006, at page 1800-199, lower left-hand column. However, to advance prosecution of the application, the specification has been amended to include that this application is a national stage application.

The specification is also objected to for not including each separate figure in the Brief Description of Drawings. By the amendment herein, the specification has been amended to include reference each Figure of drawings in the Brief Description of the Drawings.

Moreover, Fig. 6 and the specification are objected to for not including identification of the lower two figures in Fig. 6.

In response, Applicants note that the lower two figures in Fig. 6 are presently identified as 4A' and 4B'.

Accordingly, the objections to the specification should be withdrawn.

#### **Response To Rejection Under 35 U.S.C. 112, First Paragraph**

Claims 3, 4, and 7-21 are rejected under 35 U.S.C. 112, first paragraph, because the rejection contends that the deficiencies relating to the recitation of positive method steps have not been corrected.

In response, the claims have been amended herein to even more clearly denote the claimed subject matter in accordance with discussions regarding the claims during the above-noted interview. In particular, independent claim 3 even more clearly recites that

the method for detecting cholesterol in a living cell including distribution of the cholesterol in the cell, comprises contacting up to 2.0  $\mu$ M of a labeled polyethylene glycol cholesteryl ether with the living cell to bind with cholesterol present in the living cell, determining the presence and distribution of the labeled polyethylene glycol cholesteryl ether bound to cholesterol, and confirming the presence of cholesterol and distribution in the living cell based upon the determining of the presence and distribution of labeled polyethylene glycol cholesteryl ether bound to cholesterol.

Support for the amendment of claim 3 is present throughout Applicants' originally filed application including page 6, first full paragraph, and Examples 3 and 4, beginning at the bottom of page 13, and particularly page 14, lines 1-2 and 9-11, page 17, paragraph (3), page 19, first paragraph, for disclosure cholesterol distribution and labeling with up to 2  $\mu$ M fPEG-Chol.

Accordingly, this ground of rejection is no longer applicable, and should be withdrawn.

#### **Response To Rejection Under 35 U.S.C. 112, Second Paragraph**

Claims 3 and 7-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

The rejection contends that it is unclear how any detection of cholesterol is taking place in this method.

In response, Applicants submit that claims as amended herein even more clearly recite Applicants' method, whereby this ground of rejection should be withdrawn.

The rejection also questions the terminology "affinity".

In response, Applicants submit that one having ordinary skill in the art would readily understand the scope of the claims, especially in view of the description of “affinity substances” in Applicants’ originally filed application, such as Applicants’ specification at page 5, second full paragraph, and page 6, third full paragraph, where affinity substances are discussed along with how to detect such affinity substances.

Moreover, Applicants’ submit that binding of affinity substances such as biotin with avidin and streptavidin is well known to those having ordinary skill in the art, and one having ordinary skill in the art would understand the scope of claims reciting “affinity substance”. See, for example, U.S. Patent No. 5,552,276 at, for examples column 4, lines 25-46, for examples of affinity substances”.

Accordingly, this ground of rejection should be withdrawn.

#### **Response To Art Based Rejections**

(a) Claims 1, 3-5, 7, 9-12, 14-16. 19 and 20 are rejected under 35 U.S.C. 102(b) as being anticipated by Baba et al. (hereinafter “Baba”), Traffic, Vol. 2, PP. 501-512, 2001).

In this ground of rejection, the rejection contends that Baba discloses at page 509, in the left-hand column, in the “Cells and reagents” section the same composition recited by Applicants. Moreover, for the method claims, the rejection refers to page 509, left-hand column, in the “PEG-Chol treatment” section. The rejection contends that Applicants’ recited methods would be inherently performed by the method of Baba which determines the amount of PEG-Chol associated with human erythrocyte membranes.

In response, Applicants note that product claims 1, 5 and 6 have been canceled without prejudice or disclaimer of the subject matter recited therein.



Regarding the method claims, Applicants note that independent claim 3 includes that the method for detecting cholesterol in a living cell including distribution of the cholesterol in the cell, comprises contacting up to 2.0  $\mu\text{M}$  of a labeled polyethylene glycol cholesteryl ether with the living cell to bind with cholesterol present in the living cell, determining the presence and distribution of the labeled polyethylene glycol cholesteryl ether bound to cholesterol, and confirming the presence of cholesterol and distribution in the living cell based upon the determining of the presence and distribution of labeled polyethylene glycol cholesteryl ether bound to cholesterol.

In contrast to Applicants' independent claim 3, Baba is directed to the examination of the effect of a cholesterol derivative poly(ethylene glycol) cholesteryl ether on the structure/function of clathrin-coated pits and caveolae. Baba indicates that the addition of the compound to cultured cells induced progressive smoothening of the surface. At page 509, Baba discloses the determination of the amount of PEG-Chol associated with human erythrocyte membranes by incubating cells with the compound at various concentrations, washing with PBS, fixing the cells with glutaraldehyde, extracting PEG-Chol from the cells, and colorimetrically determining the concentration. Moreover Baba discloses that the amount of PEG-Chol inserted in the surface of K562 and A431 cells was estimated by using biotinyl PEG-Chol.

Baba does not disclose the various steps performed in Applicants' process. The rejection asserts that, "It is inherent in the method of Baba et al. that the bPEG-Chol was detectably labeling cell membrane cholesterol." The rejection relies upon the assertion that the discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation of the prior art's function, does not render the old composition

patentably new to the discoverer, or that claiming of a new use, new function or unknown property which is inherently present in the prior art does not necessarily make the claim patentable.

In contrast to the assertions in the rejection, the method recited in Applicant's method claims includes steps that are not practiced in the prior art, and the claims as presently amended even more clearly recite such steps that are not practiced in Baba. Therefore, Baba does not teach each and every feature recited in the Applicants' claims (including Applicants' dependent claims which further patentably define Applicants' invention), either explicitly or implicitly, whereby the rejection of record is without appropriate basis and should be withdrawn.

Still further, Applicants note that Baba discloses a phenomenon that PEG-Chol inhibited clathrin-independent endocytosis. However, the mechanism by which PEG-Chol inhibits clathrin-independent endocytosis, has not yet been fully revealed. For example, it may be possible that PEG-Chol inhibits the function of caveolin, or it may be possible that PEG-Chol acts on any other component in the raft. Therefore, Baba does not show that PEG-Chol binds to cholesterol.

Further, Baba uses PEG-Chol at a high concentration of 5 to 10 mM which induces inhibition of clathrin-independent endocytosis, and thus detection of cholesterol in cells of normal state is not possible. Namely, since dynamics of cholesterol depends on clathrin-independent endocytosis, exact dynamics of cholesterol cannot be analyzed if a high concentration of PEG-Chol is used.

As discussed above, Baba does not teach that PEG-Chol can recognize cholesterol. Further, when detection is carried out in the concentration disclosed by Baba, Applicants' recited method will apparently not be achieved.

Moreover, Applicant notes that it is an advantage of the present invention that the cholesterol detecting agent of the present invention can be used for living cells, as compared to other cholesterol agents. For example, filipin (Figs. 2A and 2B) used in Example 2 of the present application is highly toxic and cannot be used for living cells.

Accordingly, the anticipation rejection should be withdrawn.

(b) Claims 1, 3-7, 9-12, 14-16, 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Baba in view of Wu et al., U.S. Patent No. 6,005,113.

The deficiencies of Baba are noted above, such that Baba does not teach or suggest the method recited in Applicants' claims. Baba does not disclose the various steps performed in Applicants' process, and there is no reason why such steps recited in Applicants' claims would be arrived at following any disclosure in Baba. Thus, one having ordinary skill in the art would not arrive at Applicants' claimed method following any disclosure in the prior art, which method is for detecting cholesterol in a living cell including distribution of the cholesterol in the cell, comprising contacting up to 2.0  $\mu$ M of a labeled polyethylene glycol cholesteryl ether with the living cell to bind with cholesterol present in the living cell, determining the presence and distribution of the labeled polyethylene glycol cholesteryl ether bound to cholesterol, and confirming the presence of cholesterol and distribution in the living cell based upon the determining of the presence and distribution of labeled polyethylene glycol cholesteryl ether bound to cholesterol.

Moreover, the deficiencies of Baba are not overcome by Wu. Wu is merely utilized in the rejection in an attempt to establish using digoxigenin as a label in Baba. Therefore, whether or not it would have been obvious to combine the disclosures of Baba and Wu, Applicants' claimed subject matter would not be arrived at. In this regard, Applicants note that no combination of Baba and Wu, even if appropriate, would arrive at a method for detecting cholesterol in a living cell including distribution of the cholesterol in the cell, comprising contacting up to 2.0  $\mu$ M of a labeled polyethylene glycol cholesteryl ether with the living cell to bind with cholesterol present in the living cell, determining the presence and distribution of the labeled polyethylene glycol cholesteryl ether bound to cholesterol, and confirming the presence of cholesterol and distribution in the living cell based upon the determining of the presence and distribution of labeled polyethylene glycol cholesteryl ether bound to cholesterol.

If the rejection is maintained, the Examiner is respectfully requested to explicitly point out where Baba discloses each of the steps recited in Applicants' claims and/or any reason to perform such steps.

Accordingly, the rejections of record should be withdrawn with the early mailing of the Notices of Allowance and Allowability.

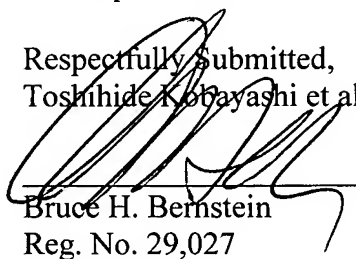
CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejections of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully Submitted,  
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